Biomaterial Interfaces Division
Room A120-121 - Session BI+AS-WeM

Microbes and Fouling at Surfaces

Moderators: David G. Castner, University of Washington, Kenan Fears, U.S. Naval Research Laboratory

8:00am **BI+AS-WeM1 Hydrophilic Polysaccharides as Building Blocks for Marine Fouling-release Coatings**, ***Axel Rosenhahn***, *V. Jakobi, X. Cao, W. Yu, T. Gnanasampanthan, R. Wanka, J. Schwarze, J. Koc,* Ruhr-University Bochum, Germany*; M. Grunze,* Heidelberg University, Germany*; J.A. Finlay, A.S. Clare,* Newcastle University, UK*; K.Z. Hunsucker, G.E. Swain,* Florida Institute of Technology

Hydrophilic building blocks like polyethylene glycols are powerful ingredients in modern fouling-release coatings as they are capable to reduce the attractive hydrophobic interactions of microbes with hydrophobic matrix materials such as acrylates, silicones or polyurethanes. We explored how polysaccharides with known antiadhesive and anti-inflammatory properties in medical applications reduce the adhesion of marine fouling organisms. Among the advantages of polysaccharides is their availability, biocompatibility and degradability. Based on previous work on hydrophilic coatings [1] we focused on well hydrated hyaluronans, alginates, chitosans and chondroitin sulfate building blocks. The response of marine organisms and the ability of such components to reduce attachment and facilitate easy removal is explored on grafted monolayers of polysaccharides [2], their amphiphilic derivatives [3], and in more complex coatings such as polysaccharide containing polyelectrolyte multilayers and hybrid polymers. Lab and field experiments will be compared and discussed in the light of the previous notion that uptake of soil particles frequently challenge hydrophilic polymers when used in the real ocean environment [4].

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[3]V. Jakobi, J. Schwarze, J. A. Finlay, K. A. Nolte, S. Spöllmann, H.-W. Becker, A. S. Clare, A. Rosenhahn, Biomacromol. 2018,19, 402-408.

[4] J. Koc, T. Simovich, E. Schönemann, A. Chilkoti, H. Gardner, G.W. Swain, K. Hunsucker, A. Laschewsky, A. Rosenhahn, Biofouling 2019, DOI:10.1080/08927014.2019.1611790

8:20am **BI+AS-WeM2 A Microfluidic Assay to Test the Adhesion of the Marine Bacterium *Cobetia Marina* Under Dynamic Shear Conditions**, ***Jana Schwarze***, *K.A. Nolte, R. Wanka, V. Jakobi, A. Rosenhahn,* Ruhr-University Bochum, Germany

Microfluidic environments with laminar flow are a useful tool to quantify attachment and removal of marine biofilm formers and cells[1–3]. We present results on the microfluidic attachment of the marine bacterium *Cobetia marina* (*C. marina*), formerly named *Halomonas marina*, as it is frequently found in marine biofilms[4,5]. To identify a suitable shear stress for the microfluidic attachment assays, the attachment behavior of *C. marina* was investigated at different shear forces on hydrophobic and hydrophilic surfaces, whereby *C. marina* tends to adhere best on hydrophobic coatings. Among the optimized assay parameters are the relevance of the growth state of *C. marina*. The optimized assay parameters will be presented as well as selected examples how coating chemistries like different self-assembling monolayers, amphiphilic alginates[6] and different thicknesses of PG coatings[7] alter the attachment of the marine bacterium *C. marina* under dynamic shear conditions.

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2. Alles, M. & Rosenhahn, A. Microfluidic detachment assay to probe the adhesion strength of diatoms. *Biofouling* (2015), 31:5, 469-480. doi:10.1080/08927014.2015.1061655

3. Nolte, K. A., Schwarze, J. & Rosenhahn, A. Microfluidic accumulation assay probes attachment of biofilm forming diatom cells. *Biofouling* (2017), 33 (7): 531-543. doi:10.1080/08927014.2017.1328058

4. Ista, L. K., Fan, H., Baca, O. & López, G. P. Attachment of bacteria to model solid surfaces' oligo(ethylene glycol) surfaces inhibit bacterial attachment. *FEMS Microbiol. Lett.* (1996), 142, 59-63. doi:10.1016/0378-1097(96)00243-1

5. Arpa-Sancet, M. P., Christophis, C. & Rosenhahn, A. Microfluidic assay to quantify the adhesion of marine bacteria. *Biointerphases* (2012), 7, 26. doi:10.1007/s13758-012-0026-x

6. Jakobi, V. *et al.* Amphiphilic Alginates for Marine Antifouling Applications. *Biomacromolecules* (2018), 19, 402-408. doi:10.1021/acs.biomac.7b01498

7. Wanka, R. *et al.* Fouling-Release Properties of Dendritic Polyglycerols against Marine Diatoms. *ACS Appl. Mater. Interfaces* (2018), 10, 34965–34973. doi:10.1021/acsami.8b12017

8:40am **BI+AS-WeM3 Biofilm Mechanics as a Mechanism for Survival on Surfaces from Medical Device to Ship Hulls**, ***Paul Stoodley***, Ohio State University **INVITED**

Bacterial biofilms are microscopic assemblages of bacterial cells usually attached to a surface and held together by a self-produced extracellular polymeric slime (EPS) matrix. Biofilms are ubiquitous in the natural environment and are highly problematic in industry and medicine where they cause corrosion, fouling, contamination and chronic medical and dental infections. The basic biology of bacterial biofilm development and strategies evolved to survive in the environment of the ancient earth are now used by the bacteria to survive on modern man-made materials. Diffusion limitation within the EPS matrix results in sharp gradients as nutrients are consumed by respiring bacteria on the periphery faster than they can diffuse in. Similarly, cell signals (molecules used to co-ordinate behavior between individual cells) and waste products, such as fermentation acids, build up in the interior of the biofilm. Biofilms are mechanically complex showing a range of behaviors from elastic solids to viscous liquids. These viscoelastic properties can facilitate survival on surfaces exposed to high shear stresses and can explain the high pressure drop and frictional losses in pipelines and ship hull fouling. However, the mechanical response may also be exploited to drive antimicrobials into the biofilm for control. The development of microenvironments combined with the structural versatility of the biofilm is the basis for the distinct biofilm phenotype as an emergent property of population of single cells and is a challenge to overcome in their control.

9:20am **BI+AS-WeM5 Dendritic Polyglycerols as Fouling-release Coatings Against Marine Hard- and Soft Fouler**, ***Robin Wanka***, Ruhr-University Bochum, Germany*; N. Aldred, J.A. Finlay,* Newcastle University, UK*; K.A. Nolte, J. Koc,* Ruhr-University Bochum, Germany*; H. Gardner, K.Z. Hunsucker, G.E. Swain,* Florida Institute of Technology*; C. Anderson, A.S. Clare,* Newcastle University, UK*; A. Rosenhahn,* Ruhr-University Bochum, Germany

Polyethylene glycol (PEG) containing coatings show outstanding antifouling properties, which is commonly assigned to their hydrophilicity and their highly hydrated nature. A structurally related but hyperbranched version are polyglycerols (PGs) that increase the spatial density of non-fouling polymer units and decrease the defect density in coatings.[1,2] So far they were successfully applied in biomedicine against attachment by pathogenic bacteria. Using a surface initiated ring opening polymerization reaction[3], we grafted dendritic PGs on surfaces. The resulting samples were characterized by spectroscopic ellipsometry, contact angle goniometry, ATR-FTIR, and by degradation experiments. The prepared surfaces show excellent protein-resistance. The fouling release properties were tested in a standardized lab assay with diatoms (*Navicula incerta)* and in a dynamic field assay[4] at the FIT test site in Florida. The initial attachment of diatoms under static conditions was similar on the PGs as compared to a hydrophobic control. However, PGs show outstanding fouling release properties. Up to 94% of attached diatoms could be removed from the coatings after the exposure to a shear stress of 19 Pa. These results were confirmed in the field assays.[5] The range of testes species was also extended to macro-fouling organisms such as zoospores of green algae (*Ulva linza)* and barnacle larvae (*Balanus amphitrite*).

(1) Wyszogrodzka, M.; Haag, R. Synthesis and characterization of glycerol dendrons, self-assembled monolayers on gold: A detailed study of their protein resistance. *Biomacromolecules* **2009**, *10*, 1043–1054.

(2) Siegers, C.; Biesalski, M.; Haag, R. Self-assembled monolayers of dendritic polyglycerol derivatives on gold that resist the adsorption of proteins. *Chemistry* **2004**, *10*, 2831–2838.

(3) Weber, T.; Gies, Y.; Terfort, A. Bacteria-Repulsive Polyglycerol Surfaces by Grafting Polymerization onto Aminopropylated Surfaces. *Langmuir* **2012**, *28*, 15916–15921.

(4) Nolte, K. A.; Koc, J.; Barros, J. M.; Hunsucker, K.; Schultz, M. P.; Swain, G. E.; Rosenhahn, A. Dynamic field testing of coating chemistry candidates by a rotating disk system. *Biofouling* **2018**, *49*, 1–12.

(5) Wanka, R.; Finlay, J. A.; Nolte, K. A.; Koc, J.; Jakobi, V.; Anderson, C.; Clare, A. S.; Gardner, H.; Hunsucker, K.; Swain, G. W. *et al.* Fouling-Release Properties of Dendritic Polyglycerols against Marine Diatoms. *ACS applied materials & interfaces* **2018,** DOI: 10.1021/acsami.8b 12017 .

9:40am **BI+AS-WeM6 Nano- and Microscale ZnO with Controllable Abundance of Surface Polarity as a Platform to Study Antibacterial Action.**, *J.M. Reeks, B. Thach,* Texas Christian University*; W. Moss,* Texas State University*; R. Maheshwari,* Texas Academy of Mathematics and Science*; I. Ali, S.M. McGillivray,* ***Yuri Strzhemechny****,* Texas Christian University

Nano- and microcrystalline ZnO is a low-cost, easy to synthesize material employed in many current and incipient applications owing to its exceptional optoelectronic, structural and chemical characteristics as well as a broad range of production techniques. Antibacterial action of ZnO is one of these applications, with a growing field of interdisciplinary research. Despite numerous and vigorous studies of the antibacterial nature of ZnO, and, in particular, the well- documented antimicrobial action of micro- and nanoscale ZnO particles, the most fundamental physical and chemical mechanisms driving this action are still not well identified. In particular, the influence of the crystal surface polarity on the antibacterial performance is largely unknown. Normally, hexagonal (wurtzite) ZnO crystals can be terminated with either charged polar (Zn or O) or electrically neutral nonpolar surfaces. In this work, we employ a hydrothermal growth protocol to produce ZnO nano- and microcrystals with tunable morphology, in particular to obtain a dependable control of the prevalent polarity of the free surfaces. This, in turn, can serve as a platform to investigate antibacterial action mechanisms in the synthesized specimens. It is reasonable to assume that one of the key phenomena behind such action is rooted in interactions between ZnO surfaces and the extracellular layers. Thus, excess charge or lack thereof, surface electronic charge traps, as well as variations in the stoichiometry at surfaces with different polarities of ZnO particles may affect interfacial phenomena with cell surfaces. It is possible therefore that the relative abundance of ZnO surfaces with different polarities could significantly influence their antibacterial action. In our studies, we produced ZnO crystals comparable in size with the bacteria employed in our assays, such as s. aureus. This was done intentionally in order to avoid cellular internalization of ZnO particles and thereby to address primarily the mechanisms involving ZnO/cell surface interface. These experiments were performed in conjunction with optoelectronic studies of ZnO crystals (photoluminescence, surface photovoltage) to characterize electronic structure and dominant charge transport mechanisms as fundamental phenomena governing antibacterial characteristics of our samples. We report on the results of these comparative studies relating predominant ZnO surface polarity with the antimicrobial action.

11:00am **BI+AS-WeM10 Patterning Bacteria at Interfaces with Bio-Inspired Vascularized Polymers***, K. Marquis, B. Chasse,* ***Caitlin Howell****,* University of Maine

Nearly all methods of introducing bioactive compounds to the surface of a substrate rely on application from above or fail over time due to depletion. In this work, we use a bio-inspired approach to deliver target molecules to an interface from below, making use of both theoretical modeling and experimental validation to rationally design customizable patterns and gradients. Mimicking the vascular systems of living organisms, networks of empty 3D-printed channels are filled with liquid containing the compound of interest, which flows through the vascular network and diffuses through the polymer, eventually reaching the substrate surface. In proof-of-principle experiments using *Escherichia coli* and *Staphylococcus epidermidis* as model organisms, we demonstrate both theoretically and experimentally that the concentration of antibiotic and duration over which it is delivered to the surface can be controlled by varying the location of the vascular channels and concentration of the antibiotic solution inside. The result is a well-defined and predictable patterned response from the bacteria growing on the surface, a first step toward developing new types of adaptive antifouling surfaces and cell culture tools.

11:20am **BI+AS-WeM11 Chemical Imaging of Root-Microbe Interactions***,* ***Vaithiyalingam Shutthanandan****, A. Martinez, R. Boiteau,* Pacific Northwest National Laboratory

Nutrient mobilization from soil minerals is critical for plant growth, particularly in marginal lands with high pH soils or low phosphate and iron availability. Rhizospheric bacteria enhance plant growth by converting root exudates such as sugars and amino acids into organic acids and chelating molecules that enhance mineral dissolution and improve the availability of nutrients such as phosphorous and iron. Hence, understanding the effect of iron availability on metabolite exchange between plant and micro-organism is crucial. The spatial proximity of bacteria to root tissue of specific composition and sites of root secretion is one key aspect of this exchange. In this work, the model grass Brachypodium and the bacteria Pseudomonas fluorescens are used as a model system for studying rhizosphere interactions that improve metal bioavailability. Brachypodium was grown under four different conditions such as: (1) + Fe, (2) – Fe, (3) + Pseudomonas + Fe, (4) + Pseudomonas – Fe. The plants were grown for 2 weeks in the hydroponic solution and removed from the system and the root samples were analyzed using Helium Ion Microscope (HIM) for spatial organization of bacteria within the rhizosphere of Brachypodium and X-ray photoelectron spectroscopy (XPS) for chemical imaging. HIM results clearly show bacteria colonies on the root surfaces. However, these colonies were populated preferentially within grooved structures along the surface of the root. We hypothesize that there are compositional differences in the surface of the root area that explain the presence of these 'hotspots'. Roots exposed with iron show larger bacteria colonies than the roots without iron content. XPS imaging measurements on these samples revealed four predominant compositional classes, lipids/lignin, protein, cellulose and uronic acid that were spatially resolved across the surface of the main root with ~10 □m resolution. Carbon and oxygen concentrations were almost constant among these samples and also constant along the individual roots. On the other hand, there is a clear variation in the concentrations of nitrogen and potassium along the root as well as among the samples. Discussion on the results and their implications will be discussed in this presentation.

11:40am **BI+AS-WeM12 Biocompatible Silver Nanoparticles-loaded Chitosan Membranes with Antibacterial Activity Produced by Directed Liquid-Plasma Nanosynthesis***,* ***Camilo Jaramillo****, A.F. Civantos, A. Mesa, J.P. Allain,* University of Illinois at Urbana-Champaign

Silver nanoparticles (Ag NPs) possess remarkable antibacterial properties that are widely recognized. The emergence of antibiotic-resistant bacteria has motivated the interest of Ag NPs as an alternative for antimicrobial protection, in a wide range of applications [1]. However, Ag NPs have also shown toxicity and low biocompatibility. In addition, their synthesis usually involves toxic compounds, further limiting their applicability as a biomaterial. Research on Ag NPs has largely focused on increasing their biocompatibility. Properties such as NPs size, dispersity, and stability have shown to play an important role on their biocompatibility [2]. Green synthesis methods that require non-toxic agents while giving control over these properties are of high interest.

Chitosan (CS) is a deacetylated derivative of chitin, a widely available polymer. Its properties include biodegradability, biocompatibility and non-toxicity, making it an attractive alternative for biomaterials. CS has been used as a bioactive coating (for proteins, drugs and antibiotics and as a stabilizing agent in the production of Ag NPs [3]. Approaches to synthesize CS-based Ag NPs include γ irradiation and sonochemical methods [4].

In this work, Directed Liquid-Plasma Nanosynthesis (DLPNS) was used to drive Ag NPs synthesis without the need of other reagents. CS membranes were used to immobilize the NPs, to explore their application as an antibacterial coating for biomaterials. The Ag NPs precursor concentration and irradiation time were used as control parameters. Surface topography and chemistry were studied by SEM, Contact Angle, XRD and EDS. Antimicrobial properties of the membranes were evaluated against gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria. Life and death assays revealed the antibacterial activity of the membranes. To study their biocompatibility and cytotoxicity, mammalian cell cultures were used. Cell viability, adhesion and metabolism were evaluated via Alamar blue and immunostaining tests. SEM images were used to assess the presence of Ag NPs in the CS matrix, and observe the bacteria and cell morphology on the surface of the membranes.

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12:00pm **BI+AS-WeM13 Multifunctional 2D MoS 2 -Based Nanoplatform for Multimodal Synergistic Inactivation of Superbugs***, **Paresh Ray**,* Jackson State University

Development of new antibacterial therapeutic materials is becoming increasingly urgent due to the huge threat of superbugs, which are responsible for more than half of a million deaths each year in this world. We will discuss our recent report on the development of a novel nanobiomaterial based on a melittin antimicrobial peptide (AMP)-attached transition metal dichalcogenide MoS2-based theranostic nanoplatform. The reported nanoplatform has a capability for targeted identification and synergistic inactivation of 100% multidrug-resistant superbugs by a combined photo thermal therapy (PTT), photodynamic therapy (PDT), and AMP process. A novel approach for the design of a melittin antimicrobial peptide-attached MoS2-based nanoplatform is reported, which emits a very bright and photo stable fluorescence. It also generates heat as well as reactive oxygen species (ROS) in the presence of 670 nm near-infrared light, which allows it to be used as a PTT and PDT agent. Due to the presence of AMP, multifunctional AMP exhibits a significantly improved antibacterial activity for superbugs via a multimodal synergistic killing mechanism. Reported data demonstrate that nanoplatforms are capable of identification of multidrug-resistant superbugs via luminescence imaging. Experimental results show that it is possible to kill only ~45% of superbugs via a MoS2 nanoplatform based on PTT and PDT processes together. On the other hand, killing less than 10% of superbugs is possible using melittin antimicrobial peptide alone, whereas 100% of methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Escherichia coli* (*E. coli*), and drug-resistant *Klebsiella pneumoniae* (KPN) superbugs can be killed using antimicrobial peptide-attached MoS2 QDs, via a synergistic killing mechanism.

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